

CLAIMS

We claim:

1. A non-naturally occurring NRSF-based zinc-finger polypeptide that differs from a naturally occurring NRSF zinc-finger polypeptide comprising at least one amino acid residue in at least one zinc finger that differs in amino acid sequence from the naturally occurring NRSF zinc-finger polypeptide, wherein the naturally occurring NRSF zinc finger polypeptide binds to a NRSE consensus sequence, and the non-naturally occurring NRSF-based zinc finger polypeptide binds to a sequence of interest but does not bind to the NRSE consensus sequence.
2. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 1, wherein the polypeptide comprises at least two zinc-fingers.
3. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 1, wherein the polypeptide is monomeric, dimeric, or multimeric.
4. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 1, wherein the polypeptide comprises one or more functional domains.
5. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 4, wherein the functional domain(s) are selected from the group comprising transcriptional activation domain, transcriptional repressor domain, transcriptional silencing domain, acetylase domain, de-acetylase domain, methylation domain, de-methylation domain, kinase domain, phosphatase domain, dimerization domain, multimerization domain, nuclear localization domain, nuclease domain, endonuclease domain, integrase domain, and resolvase domain.
6. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 5, wherein the polypeptide comprises a transcriptional activation domain.
7. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 5, wherein the polypeptide comprises a transcriptional repression domain.
8. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 5, wherein the polypeptide comprises a silencing domain.
9. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 5, wherein the polypeptide comprises either or both of the C-terminal and N-

terminal transcriptional repression domains of a naturally occurring NRSF protein.

10. The non-naturally occurring NRSF-based zinc-finger polypeptide of claim 5, wherein the polypeptide comprises an endonuclease domain.
- 5 11. A method of regulating the expression of a gene comprising contacting a non-naturally occurring NRSF-based zinc-finger polypeptide according to claim 1 with a sequence of interest in the gene, such that the expression of the gene is regulated.
12. A method of altering the structure of a nucleic acid molecule, comprising  
10 contacting a NRSF-based zinc-finger polypeptide according to claim 1 with a sequence of interest to form a binding complex, such that the structure of the nucleic acid molecule is altered.
13. A method of altering the structure of chromatin comprising contacting a non-naturally occurring NRSF-based zinc-finger polypeptide according to claim 1  
15 with a sequence of interest to form a binding complex, such that the structure of the chromatin is altered.
14. A method of cleaving a sequence of interest, comprising contacting a non-naturally occurring polypeptide according to claim 10 with a sequence of interest under conditions sufficient to cleave the sequence of interest.
- 20 15. A method of silencing of a gene, comprising contacting a sequence of interest in the gene with a non-naturally occurring NRSF-based zinc-finger polypeptide according to claim 8 to form a binding complex, wherein the gene is silenced.
16. A method of selecting a non-naturally occurring NRSF-based zinc-finger polypeptide that binds to sequence of interest, comprising:  
25 a) expressing nucleic acid libraries encoding NRSF-based zinc finger polypeptides in a polypeptide expression system, wherein the NRSF-based zinc finger polypeptides have at least one randomized amino acid position within at least one zinc finger,  
b) incubating the NRSF-based zinc finger polypeptides with the sequence of  
30 interest under conditions sufficient to form binding complexes, and  
c) selecting the NRSF-based zinc finger polypeptides that bind to the DNA sequence of interest.

17. The method according to claim 16, wherein the NRSF-based zinc finger polypeptides comprise at least 4 zinc-fingers.
18. The method according to claim 16, wherein the nucleic acid libraries encoding NRSF-based zinc finger polypeptides are expressed in a phage display polypeptide expression system.
19. The method according to claim 16, wherein the nucleic acid libraries encoding NRSF-based zinc finger polypeptides are expressed in a eukaryotic or prokaryotic polypeptide expression system.
20. The method according to claim 16, wherein the nucleic acid libraries encoding NRSF-based zinc finger polypeptides are expressed in a bacterial polypeptide expression system.
21. A method of selecting a non-naturally occurring NRSF-based zinc finger polypeptide that binds to a sequence of interest, comprising the steps of:
- a) incubating primary libraries with target site constructs under conditions sufficient to form first binding complexes, wherein the primary libraries comprise NRSF-based zinc finger polypeptides having one variable finger and at least one anchor finger having, and wherein the target site construct has one subsite with a sequence identical to a subsite of the sequence of interest, and one or more subsites with sequences to which the anchor finger(s) bind;
  - b) isolating pools comprising nucleic acid sequences encoding polypeptides, wherein said polypeptides comprise the first binding complexes;
  - c) recombining the pools to produce a secondary library;
  - d) incubating the secondary library with the sequence of interest under conditions sufficient to form a second binding complex; and
  - e) isolating nucleic acid sequences encoding NRSF-based zinc finger polypeptides, wherein the NRSF-based zinc finger polypeptides comprise the second binding complexes.
22. The method of claim 21, wherein the NRSF-based zinc finger polypeptides that comprise the second binding complexes bind to the DNA sequence of interest with high affinity and specificity.
23. A nucleic acid library encoding NRSF-based zinc finger polypeptides, wherein the NRSF-based zinc finger polypeptides comprise at least one anchor finger

with an amino acid sequence identical to a zinc finger of a naturally occurring NRSF polypeptide; and at least variable finger with at least one randomized amino acid residue.

24. A nucleic acid library encoding NRSF-based polypeptides according to claim 23,  
5 wherein the variable zinc finger is derived from one of zinc fingers 3 to 8 of a naturally occurring NRSF protein.
25. A nucleic acid library encoding NRSF-based polypeptides according to claim 20, wherein the anchor fingers have the amino acid sequence of one of zinc fingers 3 to 8 of a naturally occurring NRSF protein.
- 10 26. A nucleic acid library encoding NRSF-based polypeptides according to claim 23, wherein six amino acid residues in the variable zinc finger are randomized.
27. A nucleic acid library encoding NRSF-based polypeptides according to claim 26, wherein amino acid positions -1, +1, 2, 3, 5, and 6, numbered relative to the start of the recognition alpha helix, are randomized.
- 15 28. A DNA sequence of interest to be used in the selection of a non-naturally occurring NRSF-based zinc finger polypeptide, wherein the DNA sequence of interest comprises 10 to 24 base pairs.
29. A DNA sequence of interest to be used in the selection of a non-naturally occurring NRSF-based zinc finger polypeptide, wherein the DNA sequence of  
20 interest can be described by the consensus nucleotide sequence  
5'NNNNN(C/G)NNCNNGNNCNCNNN3' (SEQ ID NO. 13).
30. A non-naturally occurring scaffold-based zinc-finger polypeptide that differs from a scaffold zinc-finger polypeptide comprising at least one amino acid  
25 residue in at least one zinc finger that differs in sequence from the scaffold polypeptide, and wherein the scaffold polypeptide binds to a naturally occurring DNA binding site and the non-naturally occurring scaffold-based zinc-finger polypeptide binds to a sequence of interest but does not bind to the naturally occurring DNA binding site of the scaffold polypeptide.
31. The scaffold protein according to claim 30, selected from the group comprising  
30 CTCF, KS1, Evi-1, MZF, and NRSF.
32. The scaffold protein according to claim 30, wherein the scaffold protein is NRSF.

33. The non-naturally occurring scaffold-based zinc-finger polypeptide of claim 30, wherein the polypeptide is monomeric, dimeric, or multimeric.
34. The non-naturally occurring scaffold-based zinc-finger polypeptide of claim 30, wherein the polypeptide comprises one or more functional domains.
- 5 35. The non-naturally occurring scaffold-based zinc-finger polypeptide of claim 34, wherein the functional domain(s) are selected from the group comprising transcriptional activation domain, transcriptional repressor domain, transcriptional silencing domain, acetylase domain, de-acetylase domain, methylation domain, de-methylation domain, kinase domain, phosphatase domain, dimerization domain, multimerization domain, nuclear localization domain, nuclease domain, endonuclease domain, integrase domain, and resolvase domain.
- 10 36. The non-naturally occurring scaffold-based zinc-finger polypeptide of claim 35, wherein the polypeptide comprises a transcriptional activation domain.
- 15 37. The non-naturally occurring scaffold-based zinc-finger polypeptide of claim 35, wherein polypeptide comprises a transcriptional repression domain.
38. The non-naturally occurring scaffold-based zinc-finger polypeptide of claim 35, wherein the polypeptide comprises a silencing domain.
39. The non-naturally occurring scaffold-based zinc-finger polypeptide of claim 35, wherein the polypeptide comprises either or both of the C-terminal and N-terminal transcriptional repression domains of a naturally occurring NR5F protein.
- 20 40. The non-naturally occurring scaffold-based based zinc-finger polypeptide of claim 35, wherein polypeptide comprises an endonuclease domain.
- 25 41. A method of regulating the expression of a gene comprising contacting a non-naturally occurring scaffold-based zinc-finger polypeptide according to claim 30, with a sequence of interest in the gene to form a binding complex, such that the expression of the gene is regulated.
- 30 42. A method of altering the structure of a nucleic acid molecule comprising contacting a non-naturally occurring scaffold-based zinc-finger polypeptide according to claim 30 with a sequence of interest in the nucleic acid molecule to form a binding complex, such that the structure of the nucleic acid molecule is altered .

43. A method of altering the structure of chromatin comprising contacting a non-naturally occurring scaffold-based zinc-finger polypeptide according to claim 30, with a sequence of interest in the chromatin to form a binding complex, such that the structure of the chromatin is altered .
- 5 44. A method of a cleaving a sequence of interest, comprising contacting a non-naturally occurring polypeptide according to claim 40 with the sequence of interest to form a binding complex, such that the sequence of interest is cleaved.
45. A method of silencing of a gene of interest comprising contacting a non-naturally occurring scaffold-based zinc-finger polypeptide according to claim 38  
10 with a sequence of interest in the gene to form a binding complex, such that expression of the gene is silenced.
46. A method of selecting a non-naturally occurring scaffold-based zinc-finger polypeptide comprising more than three zinc fingers, that binds to a sequence of interest, comprising,  
15 a) expressing nucleic acid libraries encoding scaffold-based zinc finger polypeptides in a polypeptide expression system, wherein said polypeptides comprises at least one randomized amino acid position within at least one zinc finger,  
b) incubating said polypeptides with the sequence of interest under conditions  
20 sufficient to form binding complexes, and  
c) selecting the scaffold-based zinc finger polypeptides that bind to the sequence of interest.
47. The method according to claim 46, wherein the selected scaffold-based zinc finger polypeptides comprise at least 4 zinc-fingers.
- 25 48. The method according to claim 46, wherein the nucleic acid libraries are expressed in a phage display polypeptide expression system.
49. The method according to claim 46, wherein the nucleic acid libraries are expressed in a eukaryotic or prokaryotic polypeptide expression system.
50. The method according to claim 46, wherein the nucleic acid libraries are  
30 expressed in a bacterial polypeptide expression system.
51. A method of selecting a non-naturally occurring scaffold-based zinc finger polypeptide comprising more than three zinc fingers, that binds to a sequence of interest, comprising:

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- 5 a) incubating primary libraries with target site constructs under conditions sufficient to form first binding complexes, wherein the primary libraries comprise scaffold-based zinc finger polypeptides having one variable finger and at least one anchor finger having, and wherein the target site construct has one subsite with a sequence identical to a subsite of the sequence of interest, and one or more subsites with sequences to which the anchor finger(s) bind.
- b) isolating pools comprising nucleic acid sequences encoding polypeptides, wherein said polypeptides comprise the first binding complexes;
- 10 c) recombining the pools to produce a secondary library;
- d) incubating the secondary library with the sequence of interest under conditions sufficient to form a second binding complex; and
- e) isolating nucleic acid sequences encoding non-naturally occurring scaffold-based zinc finger polypeptides, wherein the scaffold-based zinc finger polypeptides comprise the second binding complexes.
- 15 52. The method of claim 51, wherein the second binding complexes are high affinity binding complexes.
53. A nucleic acid library encoding non-naturally occurring scaffold-based zinc finger polypeptides comprising at least four zinc fingers, wherein one zinc finger of the scaffold-based zinc finger polypeptides has at least one randomized amino acid residue, and wherein the remaining zinc fingers of the scaffold-based zinc finger polypeptide polypeptides have amino acid sequences identical to a scaffold polypeptide.
- 20 54. A nucleic acid library according to claim 53, wherein the scaffold polypeptide is selected from the group comprising CTCF, KS1, Evi-1, MZF, and NRSF.
- 25 55. A nucleic acid library according to claim 54, wherein the scaffold polypeptide is NRSF.
56. A nucleic acid library encoding scaffold-based zinc-finger polypeptides according to claim 53, wherein six amino acid residues in the variable zinc finger are randomized.
- 30 57. A nucleic acid library encoding scaffold-based zinc-finger polypeptides according to claim 56, wherein amino acid positions -1, +1, 2, 3, 5, and 6, numbered relative to the start of the alpha helix, are randomized.

58. A nucleic acid library encoding scaffold-based zinc-finger polypeptides according to claim 53, wherein six amino acid residues in the variable zinc finger are randomized.
59. A nucleic acid library encoding scaffold-based zinc-finger polypeptides  
5 according to claim 54, wherein amino acid positions -1, +1, 2, 3, 5, and 6, numbered relative to the start of the alpha helix, are randomized.
60. A nucleic acid library encoding scaffold-based zinc-finger polypeptides according to claim 55, wherein six amino acid residues in the variable zinc finger are randomized.
- 10 61. A nucleic acid library encoding scaffold-based zinc-finger polypeptides according to claim 59, wherein amino acid positions -1, +1, 2, 3, 5, and 6, numbered relative to the start of the alpha helix, are randomized.